In the Specification:

See cancellation of first set of claims as noted above.

REMARKS

Election/Restrictions

Information Disclosure Statement

See supplemental Information Disclosure Statement enclosed.

Specification:

In the Office Action, the disclosure is objected to because of duplicate copies of the claims. Applicant has complied with the Examiner's suggestion and made corrections in the claims noted above. No new matter was added.

Claim Objections:

Claim 2 is objected to for reciting a grammatically improper phase. Claim 2 has been amended to comply with the Examiner's suggestion. Claims 8-13 and claims 24-28 have been amended to overcome the Examiner's objections.

Claim Rejections Under 35 USC § 112, First Paragraph:

Claims 1-17 and 24-28 were rejected under 35 USC § 112, first paragraph, for reasons stated on pages 3-6 of the Office Action. Applicant respectfully traverses the rejection.

According to *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), there are many factors to be considered when determining whether the Specification provides enabling disclosure or whether any necessary experimentation is "undue." These factors include, but are not limited to:

(A) The breadth of the claims;
(B) The nature of the invention;
(C) The state of the prior art;
(D) The level of one of ordinary skill;
(E) The level of predictability in the art;
(F) The amount of direction provided by the inventor;
(G) The existence of working examples; and
(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

In re Wands, 858 F.2d 731, 737,(Fed. Cir. 1988).

It is believed to be improper to conclude that a disclosure is not enabling based on an analysis of only one of the above factors while ignoring one or more of the others. The Examiner's analysis must consider all the evidence related to each of these factors, and any conclusion of non-enablement must be based on the evidence as a whole. *Id* at 1404, 1407.

With regard to the above-described factors (factors A-H), the instant invention claims a dystrophin minigene consisting essentially of

(a) N-terminal domain;
(b) four to six rod repeats;
(c) an H1 domain of a dystrophin gene and an H4 domain of the dystrophin gene; and
(d) a cysteine-rich domain,

wherein the N-terminal domain is selected from a group consisting of a N-terminal domain of the dystrophin gene, a modified N-terminal domain of the dystrophin gene, and a N-terminal domain of a utrophin gene; the rod repeats are selected from a group consisting of rod repeats in the dystrophin gene, rod repeats in the utrophin gene, and rod repeats in a spectrin gene; the cysteine-rich domain is the cysteine-rich domain of the dystrophin gene or the utrophin gene.

The nature of the invention relates to the use of molecular biological techniques to create dystrophin minigenes harboring biological functions that can protect the muscle from dystrophin pathology and symptoms. In particular, dystrophin minigenes that can be carried in an adeno-associated virus (AAV) vector and their use are claimed.

In this field, the person of ordinary skill in the art typically has a Ph.D. degree and some working experience in molecular biology, protein chemistry and virology.

The state of the art is established by the periodical literature and the commonly used molecular biology lab manuals. Applicant encloses the following references: (Inui K et al, Brain Dev 1996,18:357-61; Hoffman EP, Arch Pathol Lab Med 1999;123:1050-2; Hartigan-O'Connor D et al Microsc Res Tech 2000; 48:223-38)

Methods of making dystrophin minigenes and modifying functional domains of the dystrophin mini-gene were well known in the art at the time of the invention was made. Those methods were simply PCR reactions and molecular cloning, which can be found in the most commonly used molecular biology lab manuals.

Furthermore, the present specification teaches how to construct and express functional dystrophin mini-gene in vivo using AAV vectors. The specification further provides detailed description on how to generate those functional minigenes.

As to the element of predictability, the Examiner takes a position that the practitioner of the invention must be able to predict, *a priori*, the tertiary structure of the protein encoded by a dystrophin mini-gene. However, this is not the proper viewpoint from which to assess predictability. It is not necessary for one skilled in the art to know before conducting any experimentation the exact structure of a mini-gene product having dystrophin activity to practice the instant invention. Rather, a skilled artisan only needs to expect that he can generate a number of candidate mini-genes using the information and technology disclosed in the specification and screen these mini-genes to obtain those that have the dystrophin activity.

In *Wands*, applicant screened hybridoma cell lines for production of antibody necessary to practice invention. Only 4 out of 143 hybridomas, or 2.8 percent, were proved to fall within the claims. Furthermore, antibodies that were proved to be high-affinity IgM came from only 2 of 10 fusion experiments. These statistics are viewed by the Board of Patent Appeals and Interferences as evidence that Wands' methods were not predictable or reproducible. The Court of Appeals Federal Circuit, however, concluded that the Board's interpretation of the data was erroneous. The Court recognized that practitioners of monoclonal antibody technology are prepared to screen hybridomas with desired characteristics, and that it would not require undue experimentation to obtain antibodies

needed to practice the claimed invention. The Court further concluded that the sole issue on enablement is whether it would require undue experimentation to produce requisite antibodies using applicants' method. In this regard, the court stated that the key word is "undue", not "experimentation", and found that, in an art where screening embodiments for a desired activity is expected experimentation, such screening is not undue experimentation. *Id.* at 1404.

The Examiner cited Chiu et al, Ngo et al., and Merz et al. to support the notion that "[a]t the time the application was filed, predicting any protein tertiary structure based on a protein structure was considered to be unpredictable due to significant problems in several areas." The cited references, however, all focus on the general problem in predicting the three-dimensional conformation of a protein solely based on the amino acid sequence of the protein. In contrast, a large body of information was available for the structure-function relationship of dystrophin protein at the time the instant application was filed. The N-terminal structure (the actin-binding domain) of dystrophin and utrophin had been solved crystallographically and the structure and functional similarities were well documented (Keep NH et al., J Mol Biol., 285:1257, 1999; Keep NH et al., Struct. Fold. Des., 7:1539, 1999; and Moores CA et al., J. Mol. Biol., 297:465, 2000, Fiona LM et al, Structure, 8:481-491, 2000). As an added proof, Fabb SA et al (Fabb SA et al, Human Mol. Genet., 7:733-741, 2002) has recently built a functional mini-gene containing only one of the two acting-binding sites in the dystrophin N-terminus, according to the X-ray crystallographic data in the literature before this invention. The sequence-structure relationship of the C-terminus,

particularly the cysteine-rich domain of dystrophin and related proteins had also been well established (Blake DJ et al., TIBS, 20:133-135; 1995, Huang X et al, Nat. Struct. Biol., 7:634-638, 2000). The protein structure of the central rod domains was also extensively studied, and its coiled-coil unit repeat structure was predicted by the primary amino acid sequences and directly visualized under the EM (Kahana, E et al, Cell Motility and the Cytoskeleton, 36:246-252, 1997, Kahana, E et al, J. Mol. Biol. 235:1271-1277, 1994). Kahana et al observed that the correctness of conformation and phasing of the rod repeat units could determine the stability of the protein (Kahana, E et al, J. Mol. Biol. 235:1271-1277, 1994). In this invention, a variety of combinations of rod repeats were spliced together in the mini-gene constructs according to previously predicted structure in the literature. The mini-gene protein products were stable and functional in the in vivo studies in mouse muscles, supporting the notion that the rod repeat structures were highly predictable. Finally, a large number of point mutations in the dystrophin gene had been studied extensively (see Amalfitano A et al., Structure and mutation of the dystrophin gene. In: Dystrophin: Gene, Protein and Cell Biology, edited by Brown et al. Cambridge Univ. Press, 1997, 1-26). As an added proof, Derek J et al in a comprehensive review article has summarized the structure and function similarities between dystrophin and utrophin, of which the majority of the publications generated and cited were prior to this invention (Derek J et al, Physiol. Review, 2002, 82:291-329), again supporting the basis of the invention.

With guidance as to structure and function (with respect to the dystrophin gene) provided by the inventor and the literature, as well as the general knowledge of how to

assess the various functions of a peptide provided by the state of the art, one skilled in the art could reasonably determine the type and position of modification within the N-terminal, the H1 domain, or the H4 domain of the dystrophin gene. The various dystrophin mini-gene constructs with modified N-terminal and/or modified H1 or H4 domains may be created using standard molecular biology techniques, and tested in either in vitro or in vivo settings without undue experimentation.

Taken together, applicant respectfully submits that, based on the evidence regarding each of the above factors, the Specification, at the time the application was filed, would have taught one skilled in the art how to make and/or use the full scope of the claimed invention without undue experimentation.

It is believed that the grounds for this rejection have been obviated, and therefore, the rejection under 35 U.S.C. § 112, first paragraph, for alleged lack of enabling disclosure, should be withdrawn.

Claim Rejections Under 35 USC § 112, Second Paragraph:

Claims 8-13 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner stated that the term "substantially complementary" in claims 8-13 is not defined. The Examiner also indicated that dependent claims 24-28 should state "the nucleotide sequence of claim.." instead of "a nucleotide sequence of claim."

Applicants have deleted the word "substantially" from claims 8-13. Claims 24-28 have been amended as suggested by the Examiner. Accordingly, it is believed that the grounds for this rejection have been obviated, and may properly be withdrawn.

Claim Rejections Under 35 USC § 102

Claims 1 and 2 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Koenig et al. Koenig et al. teaches the full length human dystrophin cDNA.

Applicant respectfully submits that claims 1 and 2, as amended, are directed to dysdrophin minigenes consisting essentially of a N-terminal domain; four to six rod repeats; an H1 domain of a dystrophin gene and an H4 domain of the dystrophin gene; and a cysteine-rich domain. Since the full length human dystrophin gene contains 24 rod repeats while the minigenes claimed in the instant invention contain only four to six rod repeats, Koenig et al. does not anticipate the instant invention.

Claims 1-3, 5-14, 16, and 24-28 further stand rejected under 35 U.S.C. § 102(a) as being anticipated by Takeda. Takeda et al. teaches construction of dystrophin minigenes of 4.5 kb or smaller. The minigenes may contain an N-terminal domain, H1 and H4, rod-repeats of various numbers, and a cysteine rich domain.

For anticipation under 35 U.S.C. § 102, the reference "must teach every aspect of the claimed invention either explicitly or implicitly. Any feature not directly taught must be inherently present." (MPEP §706.02, Rejection on Prior Art [R-1]). The Federal Circuit has held that prior art is anticipatory only if every element of the claimed invention is

disclosed in a single item of prior art in the form literally defined in the claim *(Jamesbury Corp. v. Litton Indus. Products*, 756 F.2d 1556, 225 USPQ 253 (Fed. Cir. 1985); *Atlas Powder Co. v. du* Pout; 750 F.2d 1569, 224 USPQ 409 (Fed. cir. 1984); *American Hospital Suppl v. Travenol Labs*, 745 F.2d 1, 223 USPQ 577 9Fed. Cir. 1984)

The instant invention discloses dystrophin minigenes that are functional in vivo. The specification clearly demonstrated that mdx muscles expressing the dystrophin minigene of the instant invention are fully protected against damage caused by muscle activity and are not morphologically different from normal muscle. In contrast, Takeda does not show any valid evidence that their minigenes are indeed functional in vivo. In the specification, Takeda demonstrates that mdx muscles expressing the dystrophin minigene ΔdysM3 also stain positive for β-dystroglycan, α-sarcoglycan and α1-syntrophin (Yuasa et al., Figure 4). It therefore concludes that the expression of the dystrophin minigene ΔDysM3 results in the formation of dystrophin complexes on the muscle membrane. However, the formation of dystrophin complex is necessary but insufficient to render protective effects against dystrophy phenotype as shown by Cox et al (Cox, et al, Nat. Genet. 1994, 8:333-339). Takeda fails to demonstrate that the protective effect observed in AxCAΔDysM3 infected muscles is actually resulted from the expression of the minigene DysM3. In fact, further studies preformed by the same Japanese group has revealed that adenoviral vectors induces immune response, which up-regulates utrophin and results in mitigation of muscle pathology in mdx mice (Yamamoto et al., Human Gene Therapy 11:669, 2000). As demonstrated by Takeda group in recently published articles, the protection effect in mdx

muscle can be achieved by infecting the muscles with an adenoviral vector carrying the β-galactosidase (lacZ) reporter gene alone (Yamamoto et al., Human Gene Therapy 11:669, 2000). This adenovirus LacZ vector was co-injected with the adenovirus vectors containing the dystrophin minigenes into the mdx muscle in the earlier study reported by Takeda's group. Therefore, the therapeutic effects of the minigenes might be artifacts of adenovirus infection that triggered immune response and the production of cytokines such as IL-6, which up-regulated dystrophin homologue utrophin expression. Tekeda group recently further demonstrated that direct injection of IL-6, a cytokine induced by adenoviral infection could up-regulate utrophin and achieve therapeutic effects in mdx dystrophic muscle (Fujimori et al., Human Gene Therapy 13:509, 2002). These results suggest that the functional protection observed by Takeda et al. with the dystrophin minigenes containing less than four central rod repeats is an artifact from the adenoviral vector itself. The present inventor Xiao has tested the minigene DysM3 obtained from Takeda group and found it was not functional when packaged in AAV vector and injected into the mdx leg muscle (Wang, et al PNAS, 2000, 97:13714-13719).

Accordingly, Applicant respectfully submits that Takeda et al. does not teach a functional dystrophin minigene and therefore, does not anticipate the functional minigenes of the instant invention. Reconsideration of the 35 U.S.C.§ 102 rejection is respectfully requested.

Claim Rejections Under 35 USC § 103

Claims 1, 3, 8 and 14-17 stand rejected under 35 USC § 103(a) as being unpatentable over Takeda et al. taken with Li et al. for reasons stated on page 9 of the Office Action. Specifically, the Examiner alleges that, although Takeda does not teach a rAAV comprising a dystrophin mingene operatively linked to a CMV promoter, Li teaches the use of CMV promoter in the context of rAAV.

When applying 35 U.S.C. § 103, the Examiner is required to adhere to the following tenets of patent law: (1) The claimed invention must be considered as a whole; (2) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination; (3) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and (4) Reasonable expectation of success is the standard with which obviousness is determined. (MPEP, 2141.01).

Accordingly, the CAFC in *In re Sang Su Lee* states teaching of references can be combined only if there is some suggestion or incentive to do so. *In re Sang Su Lee*, (Fed. Cir. January 18, 2002) (quoting *Acs Hosp. Sys., Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577 (Fed. Cir. 1984). Particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed. *Id.* Furthermore, the Court states even when the level of skill in the art is high, the Board must explain the reasons one of ordinary skill in the art

would have been motivated to select the references and to combine them to render the claimed invention obvious. *Id.*

First, Takeda teaches the construction of dystrophin minigenes having one to three rods, while the instant invention discloses minigene constructs having four to six rods. For this reason alone, the instant invention is distinguishable and is not obvious from Takeda. Moreover, it should be noted that the three-rod dystrophin minigenes of Takeda are already very large (4.2 kb). Therefore, they cannot be packaged with a CMV promoter (700 bp) and a polyA signal (200 bp). Based on the teachings of Takeda, it would be impossible to create a functional dystrophin minigene that contains three or more rod repeats, a CMV promoter and polyA sequences, and is still within the package limit of rAAV (5 kb). Thus, it is not desirable to use CMV promoter in Takeda constructs having three rods in the context of rAAV. For the same reason, it is also not desirable to use more than three rods in Takeda, because each rod is encoded by approximately 250 bp of DNA and it would make the minigene even larger and unfit for rAAV packaging. Therefore, Takeda does not provide any motivation to combine the minigene with a CMV promoter, nor does it provide any reasonable expectation of success. Importantly, the instant invention describes a novel and unobvious strategy to accommodate more rod domains (four to six) by deleting almost the entire very C-terminal region (approximately 800 bp) of the dystrophin, which proves to be non-essential by the inventor and makes more space for the addition of extra central rod domains to obtain functionality for the minigenes. However, this very C-terminal region is present in all of Takeda's minigenes, some of which are already too large to be packaged into

AAV vectors coupled with CMV promoter and a polyA signal. Without the deletion of the 800 bp or so C-terminal fragment, it is technically impossible for Takeda's constructs to accommodate more than three central rod domains, whereas more than three rods are apparently required for minigenes to be functional in protecting the dystrophic muscles (Harper SQ et al, Nat. Med. 2002, 8:253-261). This is a very important distinction between the instant invention and Takeda's. Accordingly, Applicant respectfully submits that the Examiner has failed to establish a prima facie case of obviousness. Reconsideration of the 35 USC § 103(a) rejection is respectfully requested.

CONCLUSION

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance.

Copies of the papers noted above are enclosed.